

Draft Genome Sequence of *Herbaspirillum lusitanum* P6-12, an Endophyte Isolated from Root Nodules of *Phaseolus vulgaris*

Vinicius Almir Weiss,^a Helisson Faoro,^a Michelle Zibbetti Tadra-Sfeir,^a Roberto Tadeu Raittz,^b Emanuel Maltempi de Souza,^a Rose Adele Monteiro,^a Rodrigo Luis Alves Cardoso,^a Roseli Wassem,^a Leda Satie Chubatsu,^a Luciano Fernandes Huergo,^a Marcelo Müller-Santos,^a Maria Berenice Reynaud Steffens,^a Liu Un Rigo,^a Fábio de Oliveira Pedrosa,^a and Leonardo Magalhães Cruz^a

Department of Biochemistry and Molecular Biology, Federal University of Paraná, Curitiba, Paraná, Brazil,^a and Technological and Professional Education Sector, Federal University of Paraná, Curitiba, Paraná, Brazil^b

Herbaspirillum lusitanum strain P6-12 (DSM 17154) is, so far, the only species of *Herbaspirillum* isolated from plant root nodules. Here we report a draft genome sequence of this organism.

Herbaspirillum lusitanum strain P6-12 was isolated from root nodules of *Phaseolus vulgaris* plants in northeastern Portugal (7). Members of the genus *Herbaspirillum* are nitrogen-fixing betaproteobacteria capable of endophytically colonizing cereals of economic relevance (3, 5).

The genome sequence of *H. lusitanum* P6-12 was determined by using a combination of fragment and mate-paired libraries on a SOLiD4 sequencer (Life Technologies), producing a total of 4,460,595 fragment and 107,836,914 mate-paired reads 50 bp in length. These libraries were used for independent *de novo* genome assembly using Velvet v.1.2.03 (8). Gap closure was achieved by combination of the two assemblies.

The draft genome of *H. lusitanum* P6-12 contains 37 scaffolds and has an estimated size of 4.9 Mb with a coverage of 214-fold and 60.2% G+C content. Automatic annotation using RAST (2) revealed 5,240 open reading frames covering 84% of the chromosome, 38 tRNAs, and a single 16S-23S-5S rRNA operon. This annotation was curated by using an in-house-developed platform named GAAT (Genome Analysis and Annotation Tool) available at www.genopar.org.

H. lusitanum was originally determined to be a nitrogen-fixing bacterium by means of pellicle formation on nitrogen-free medium and *nifD* gene amplification (7). However, no nitrogen-fixing (*nif*) genes were found in the genome. Furthermore, in our study, this strain was incapable of reducing acetylene in semisolid medium. Type I and II protein secretion systems were found. The type III secretion system, suggested to be involved in plant-bacterial interaction and present in the endophytes *H. seropedicae* SmR1 and *H. rubrisubalbicans* M1 (4), is absent from the *H. lusitanum* P6-12 genome. A *nodD*-like gene is present, although the nodulation (*nod*) genes were not found. The lack of *nif* and *nod* genes suggests that *H. lusitanum* is an opportunistic bacterium capable of colonizing root nodules, as well as other plant tissues. *H. lusitanum* P6-12 has the complete Entner-Doudoroff, pentose phosphate, and tricarboxylic acid cycle pathways. All of the genes coding for the Embden-Meyerhof-Parnas pathway are also present, although it lacks the classical 6-phosphofructokinase (EC 2.7.1.11) gene, as in *H. seropedicae* SmR1. An interesting finding is the presence of a gene coding for a protein very similar (83%) to the large ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) subunit, a key enzyme involved in photosynthetic carbon fixation (6). RuBisCO-like proteins have been reported in

other heterotrophic bacteria (e.g., *Bacillus subtilis*) and were suggested to participate in a methionine salvage pathway (1). The gene coding for 1-aminocyclopropane-1-carboxylate (ACC) deaminase was found, indicating probable contributions to plant development under stress conditions. Finally, the lack of *nif* genes raises questions regarding the ability of *H. lusitanum* P6-12 to fix nitrogen. Further analysis of the *H. lusitanum* P6-12 genome will help to improve the understanding of how bacteria may associate and interact with plants.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under accession no. [AJHH000000000](http://ajhh000000000). The version described in this paper is the first version, [AJHH010000000](http://ajhh010000000).

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REFERENCES

- Ashida H, Saito Y, Nakano T, Tandeau de Marsac N. 2008. RuBisCO-like proteins as the enolase enzyme in the methionine salvage pathway: functional and evolutionary relationships between RuBisCO-like proteins and photosynthetic RuBisCO. *J. Exp. Bot.* 59:1543–1554.
- Aziz RK, Bartels D, Best AA, Dejongh M. 2008. The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* 9:75. doi: 10.1186/1471-2164-9-75.
- James EK, Olivares FL, Baldani JJ, Döbereiner J. 1997. *Herbaspirillum*, an endophytic diazotroph colonizing vascular tissue in the leaves of *Sorghum bicolor*. *J. Exp. Bot.* 48:785–798.
- Monteiro RA, Balsanelli E, Tuleski T, Faoro H. 2012. Genomic comparison of the endophyte *Herbaspirillum seropedicae* SmR1 and the phytopathogen *Herbaspirillum rubrisubalbicans* M1 by suppressive subtractive hybridization and partial genome sequencing. *FEMS Microbiol. Ecol.* 80: 441–451.
- Pedrosa FO, et al. 2011. Genome of *Herbaspirillum seropedicae* strain

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Address correspondence to Leonardo Magalhães Cruz, leonardo@ufpr.br.

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- SmR1, a specialized diazotrophic endophyte of tropical grasses. *PLoS Genet.* 7:e1002064. doi:10.1371/journal.pgen.1002064.
6. **Tabita FR, Satagopan S, Hanson TE, Kreeb NE.** 2008. Distinct form I, II, III, and IV RubisCO proteins from the three kingdoms of life provide clues about RubisCO evolution and structure/function relationships *J. Exp. Bot.* 59:1515–1524.
 7. **Valverde A, Velazquez E, Gutierrez C, Cervantes E.** 2003. *Herbaspirillum lusitanum* sp. nov., a novel nitrogen-fixing bacterium associated with root nodules of *Phaseolus vulgaris*. *Int. J. Syst. Evol. Microbiol.* 53:1979–1983.
 8. **Zerbino DR, Birney E.** 2008. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res.* 18:821–829.